



# Cells in Tight Spaces: The Role of Cell Shape in Cell Function

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# Cells in tight spaces: the role of cell shape in cell function

Jagesh V. Shah<sup>1,2</sup>

<sup>1</sup>Department of Systems Biology, Harvard Medical School, and <sup>2</sup>Renal Division, Brigham and Women's Hospital, Boston, MA 02115

In this issue, Pitaval et al. (2010. *J. Cell Biol.* doi:10.1083/jcb.201004003) demonstrate that cell geometry can regulate the elaboration of a primary cilium. Their findings and approaches are part of a historical line of inquiry investigating the role of cell shape in intracellular organization and cellular function.

How is the internal organization of a cell influenced by its physical environment, and how does this dictate cell function? Since the earliest observations of cells, their tremendously diverse shapes have precipitated an equally large number of theories connecting geometry to function. Extended forms, as seen in some protozoa or vertebrate sperm, are thought to reduce drag for swimming motion. The cuboidal, brick and mortar-like formation of epithelia suggests a strong barrier function. The many teleological explanations, however, often remain untested. The direct manipulation of shape and subsequent observation of a cellular response is critical to validate these hypotheses. In this issue, Pitaval et al. investigate how controlling cell shape can regulate the cytoplasmic reorganization required to elaborate a primary cilium. Their use of confinement to impose a specific geometry has a long history in cell biology, starting with manipulations of large single-celled animal embryos to modern-day technologies for microscale control of a single microscopic cell. Many of these manipulations have resulted in observations that have revealed an enduring treasure trove of interesting research questions.

## Geometric control of cell division

The earliest uses of confinement were performed using embryos from amphibians and sea invertebrates (circa 1890; Pflüger, 1884; Driesch, 1893; Hertwig, 1893). These organisms produce fertilized embryos that can be stripped of extracellular membranous materials to yield a single cell on the order of a hundred micrometers up to several millimeters in diameter. At this length scale, cells can be observed with a simple microscope and manipulated with relatively “macroscopic” instruments. These eggs provide perfect specimens for observing early developmental processes, particularly the regulation of mitotic orientations that play a role in embryonic patterning. The question of how these orientations are established and coordinated

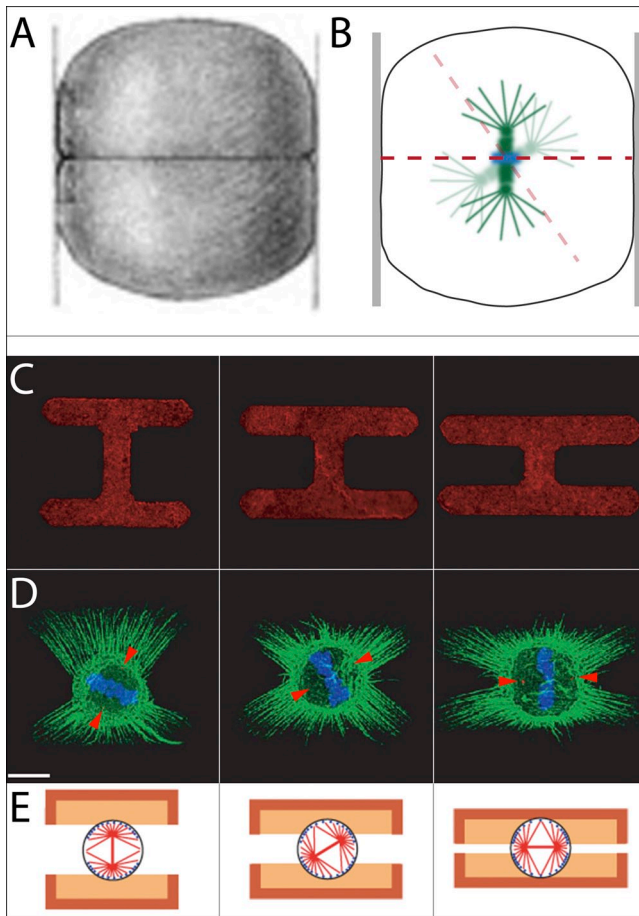
led a number of late 19th-century scientists to deform early embryos and observe the effect on cell division. Investigations by Hertwig and others (Pflüger, 1884; Driesch, 1893; Hertwig, 1893) used plates and glass capillaries to confine embryos and manipulate their shape. They observed that the division plane in compressed embryos was always perpendicular to the long axis of the cell (Fig. 1 A), and always bisected the cell. What emerged from this line of study was termed Hertwig's Rule: the mitotic spindle bisects the cell perpendicular to its longest axis. Later work by Rappaport (1961) used sand dollar eggs and dexterous manipulation to reaffirm Hertwig's rule and begin elucidating factors underlying cleavage plane specification. How a small mitotic apparatus is able to orient itself in a large cytoplasm, however, remains poorly understood, although a number of theories to explain Hertwig's rule have been recently proposed (Fig. 1 B; Grill and Hyman, 2005; von Dassow et al., 2009; Wühr et al., 2009).

The ability to control cell shape in mammalian cell culture seems a daunting task in comparison to the macroscopic manipulations of large embryos. Even so, there have been heroic efforts at manipulating single cells with “micro-instruments” (Nicklas, 1967; O'Connell and Wang, 2000). Technologies to regulate the cellular environment at the micrometer scale through so-called soft lithography methods now provide robust, reproducible, and even high-throughput approaches to regulate cell shape in an individual mammalian cell. Methods for regulating cells shape were popularized by George Whitesides, Don Ingber and their colleagues using a simple technique to pattern extracellular matrix and define adherent cell shape (Singhvi et al., 1994; Chen et al., 1997).

Théry et al., (2007) exploited these methods to pattern adhesive islands of varying geometries that accommodate a single cell (Fig. 1 C). They asked if by specifying interphase adhesion they could determine mitotic orientation. Through observations of cells grown on a wide range of shapes, they could relate adhesion geometry to spindle orientation (Fig. 1 D). In fact, Théry et al. (2007) were able to derive a simple mathematical relationship to predict mitotic orientation from the shape of the adhesive surface (Fig. 1 E). From this study, the authors proposed that the actin-rich retraction fiber that connects

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Correspondence to Jagesh V. Shah: Jagesh\_Shah@hms.harvard.edu



**Figure 1. Cell shape controls mitotic orientation.** (A) Drawing by O. Hertwig of the confined frog embryo after its first division illustrating Hertwig's rule. The image was scanned by Google Docs from Hertwig (1893). (B) Without apparent interactions with the cell boundary, we might expect the small mitotic spindle in a large cell to position randomly, but it orients according to Hertwig's rule. This image was adapted from Wühr et al. (2009). (C and D) Micropatterned fibronectin in various geometries results in well-defined mitotic orientations (C) determined by actin-rich retraction fibers (D; green). blue, DNA; arrowheads, positions of spindle poles. (E) Computational modeling predicts the expected orientation based on adhesion pattern shape. C–E are reprinted from Théry et al., 2007 with permission from Nature Publishing Group.

the extracellular matrix with the cellular cortex could convey this geometric memory. This study and others like it have spawned the hunt for the molecular pathways that determine cell division orientation in single cells and within developing tissues (Gibson and Gibson, 2009).

#### Geometric confinement determines cytoplasmic organization

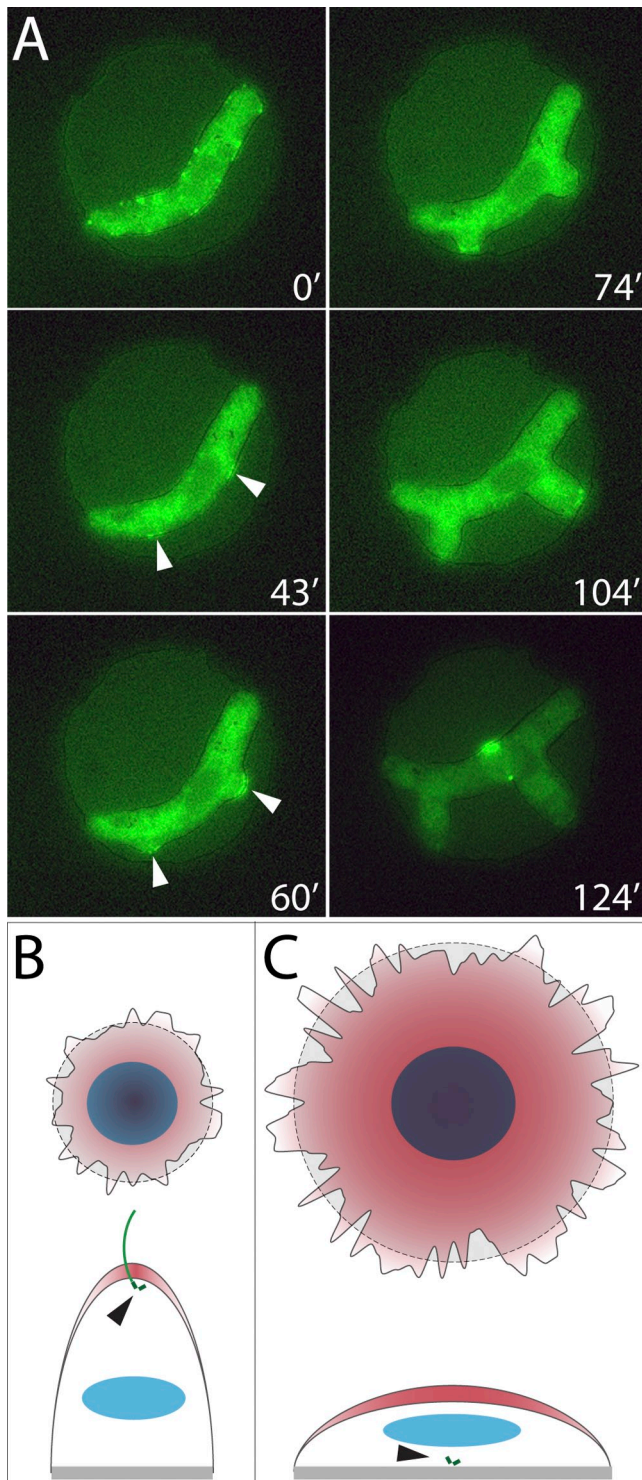
As we might expect, nondividing cells are also affected by their shape. A cell's shape in single-celled organisms, developing embryos, and terminally differentiated tissues is often adapted for some aspect of its local function. But how does a cell's shape influence its physiology outside of mitosis? The use of microscale technologies to directly manipulate adhesion geometry permits exquisite control of cell shape. In addition to patterning the extracellular domain of adherent cells, related techniques can be used to build channels and wells that are the same size

as single cells in suspension. A brilliant use of this technology has been made in the work of the laboratories of Tran and Chang (Piel and Tran, 2009; Terenna et al., 2008; and Minc et al., 2009), investigating polarized growth in the fission yeast *Schizosaccharomyces pombe*.

Fission yeast is unique in the canon of model organisms because it undergoes essentially one-dimensional growth (Mitchison and Nurse, 1985). This growth occurs through the addition of materials primarily at cell ends. As such, a long-standing problem has been how cells restrict deposition of the materials of growth to the ends and not at intermediate sites. A number of previous studies, including those of Tran and Chang, had identified the interphase microtubule array as the key polarizing structure (for review see Piel and Tran, 2009). Proteins are trafficked along these bundles to create polarized sites of cell growth at cell ends. Loss of these proteins or microtubules caused gross defects in polarized cell growth. To directly assess the role of the microtubule array, both groups used microfluidic chambers to confine wild-type cells so that growth caused the cells themselves to bend (Fig. 2 A). The bending, in turn caused the stiff and straight interphase microtubule bundles to direct polarization cues to sites not at the cell ends (Fig. 2 A; Terenna et al., 2008; Minc et al., 2009). Under certain circumstances, these mislocalized cues resulted in the branched growth of the single cells (Fig. 2 A). Through the use of microscale confinement, a shape change diverted a polarization cue, demonstrating the link between localized protein deposition and polarized growth. Studies in these confining geometries, particularly in microbes and in vitro systems, are providing even more connections between shape and growth (Nédélec et al., 1997; Moseley and Nurse, 2010).

Pitaval et al. (2010) take advantage of the micropatterning method to control cell shape, and demonstrate a link between cellular spreading and ciliogenesis. Primary cilia are organelles elaborated from the mother centriole at the apical surface of cells. This solitary microtubule-based structure is present on many cells types and is responsible for a diverse set of functions (Gerdes et al., 2009). How cilia are built is an active area of research (Seeley and Nachury, 2010), but it is known that an essential step is the migration of the centriole pair to the apical surface of a polarized cell and subsequent docking to the actin-rich cortex. The conditions required for this journey remain unclear. Pitaval et al. (2010), following previous work by Théry et al. (2006) investigating cytoplasmic organization under confinement, sought out to investigate the role of confinement in producing cilia. Through the use of patterned extracellular matrix, they confined the shape of growth-arrested cells and tested their ability to produce a primary cilium. Cells plated on small islands underwent cell cycle exit and produced primary cilia much like their unconfined counterparts (Fig. 2 B). As the island area was increased, cells became more and more spread out. Although these cells exit the cell cycle, they were unable to elaborate a primary cilium; when they did, these cilia were consistently shorter in length (Fig. 2 C). Detailed immunofluorescence imaging revealed that the centriole was often "trapped" below the nucleus and had not migrated to the apical surface. Perhaps most surprising was that the release of





**Figure 2. Cell shape controls cytoplasmic organization.** (A) Time-lapse imaging of a deformed fission yeast cell in a microwell (30  $\mu$ m in diameter) results in the local accumulation of bud6-3xEGFP, a polarity marker (43', white arrowheads), and subsequent cell growth from the ectopic site (60'–124'; experiment performed in a *cdc25-22 tea1 $\Delta$*  background). Reprinted from Minc et al., 2009 with permission from Elsevier. (B) Cellular confinement on a small extracellular matrix island results in low cortical tension (red), migration of the centriole pair (arrowhead) above the nucleus (blue), and the elaboration of a primary cilium. (C) Cell spreading on a larger island results in higher cortical tension (red), centriole trapping below the nucleus (arrowhead), and the inability to form a primary cilium. Adapted from Pitaval et al. (2010).

cortical tension via actin depolymerization, Rho kinase inhibition, or myosin II inhibition permitted the migration of the centriole pair to the apical surface and the elaboration of a cilium. Together, the data point to a “tug-of-war” between cortical tension that acts to keep cells adhered to the surface, and centriole migration and cilium extension. On small islands, the cilium wins, whereas on the larger islands the actin cortex dominates. The question of how this occurs will be an interesting one going forward.

A cell's shape is defined by a global observation: round, cuboidal, polygonal, etc. But the mechanism by which a cell can translate its shape into an intracellular signal is a local, molecular measurement. This defines the historical search for the elusive transduction machinery that integrates local cues from a cell's shape into its physiology. Combining the technologies of confinement with the modern toolbox of cell biology will be indispensable in understanding the diversity of cell shapes we see in nature.

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